Simvastatin Therapy Prevents Brain Trauma-Induced Increases in β-Amyloid Peptide Levels

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Elevations in β-amyloid peptide (Aβ) levels after traumatic brain injury (TBI) may confer risk for developing Alzheimer's disease in head trauma patients. We investigated the effects of simvastatin, a 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor, on hippocampal Aβ burden in a clinically relevant head injury/intervention model using mice expressing human Aβ. Simvastatin therapy blunted TBI-induced increases in Aβ, reduced hippocampal tissue damage and microglial activation, and improved behavioral outcome. The ability of statins to reduce post-injury Aβ load and ameliorate pathological sequelae of brain injury makes them potentially effective in reducing the risk of developing Alzheimer's disease in TBI patients.

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Traumatic brain injury (TBI) is the leading cause of death and disability in people of working age in the United States.[1] The significant social and economic burdens of TBI[2] are further amplified by the long-term consequences that include increased risk for development of Alzheimer's disease (AD).[3] The pathological links between TBI and AD are not well understood but may involve general structural damage, resulting in decreased cognitive reserve,[4] combined with β-amyloid peptide (Aβ) accumulation in brain parenchyma.[5,6] Immediately after TBI, levels of Aβ increase in brain tissue,[7] and Aβ forms neuropil deposits reminiscent of amyloid plaques that develop in AD.[6,8–11] The progressive increase in brain Aβ levels can be detrimental for brain function, as exemplified in AD, where increased Aβ load correlates with cognitive decline.[12] Similarly, Aβ accumulation after TBI could contribute to the conversion of acute injury responses to a chronic injury condition.[5,6,13] These observations suggest that current TBI therapies, aimed at preventing acute neuronal death, axonal damage, and synaptic dysfunction, could benefit from including strategies to attenuate injury-induced increases in Aβ, to delay or prevent Aβ-related pathology in the chronic course. Ideally, such a therapeutic strategy should have the dual effect of improving neurological recovery after brain injury and reducing the risk for development of AD later in life.

3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), currently in use clinically to reduce high peripheral cholesterol levels,[14] have therapeutic potential in AD.[15,16] These drugs also proved to be beneficial in animal models of brain injury including ischemia,[17] stroke,[18,19] and TBI.[20] The main goal of this investigation was to determine whether statin treatment given at a clinically relevant time point after TBI, and at a therapeutically effective dosage, would blunt postinjury increases in Aβ peptide in the hippocampus. We applied controlled cortical impact (CCI) injury[21] to $APP^{NLh/NLh}$ mice expressing human Aβ,[22] to examine the effect of simvastatin therapy on human Aβ levels, as well as on necrotic lesion size, synaptophysin immunoreactivity, microglia activation, and functional outcome. We reasoned that if statin intervention was effective in reducing Aβ levels (and therefore, potentially, the risk for AD) in a clinically and therapeutically relevant fashion, it could prove promising as a therapeutic treatment in human brain injury.

Materials and Methods

Animals and Husbandry

The University of Pittsburgh Institutional Animal Care and Use Committee approved all investigative procedures. Male 90-day-old $APP^{NLh/NLh}$ mice were used in this investigation. The $APP^{NLh/NLh}$ mouse is a nontransgenic model, with the human Aβ coding sequence "knocked-in" to the endogenous amyloid precursor protein (APP) gene (using site-based mutagenesis), and with the Swedish FADK670N/M671L mutation to achieve production of detectable levels of human Aβ.[22] Unlike traditional APP transgenic mouse models, $APP^{NLh/NLh}$ mice do not have continuous APP overexpression, because the APP gene is under control of its endogenous promoter. No spontaneous deposition of Aβ into plaques was observed in these mice examined as old as 22 months[23]; however, concentrations of human Aβ peptides are detectable by enzyme-linked immunosorbent assay (ELISA) methods.[22] Thus, the adult $APP^{NLh/NLh}$ mice are a useful rodent model to study injury-induced changes in APP and human Aβ, as well as the effects of therapies designed to manipulate levels of these proteins in vivo. Mice were maintained on a 12-hour light/dark cycle with free access to food and water before experimentation.

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Experimental Paradigm

Mice were randomly assigned to experimental groups (n = 5–6 mice/group). Experimental groups included: (1) injured mice receiving either simvastatin (Merch & Co., Inc., Whitehouse Station, NJ; 3mg/kg) or (2) vehicle (3% methylcellulose) treatment; (3) sham-operated mice receiving either simvastatin or (4) vehicle treatment; and (5) naïve (untreated) mice. Our choice of dose was guided by a previous study that reported beneficial outcomes of simvastatin intervention in a rat model of stroke.[19] Simvastatin or vehicle was administered daily, starting 3 hours after CCI, by oral gavage. Hippocampal Aβ peptide levels were quantified at 3 and 7 days after injury, the time points of sustained increases in $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$ after CCI in this mouse model,[24] whereas behavioral testing and histological-immunohistochemical analyses were conducted at 14 days after injury.

Controlled Cortical Impact Injury

Mice were subjected to CCI as described previously.[25] In brief, anesthesia was induced with 4% isoflurane in 50% N_2O/O_2 (Anaquest, Madison, WI), and mice were maintained on 2% isoflurane in 66% N_2O/O_2 for the duration of the surgical procedure (approximately 30 minutes). All mice undergoing surgery (ie, injured and sham) received the same anesthesia protocol to obviate potential confounds caused by isoflurane-induced changes in Aβ.[26] Mice were placed on a heating pad in a stereotaxic apparatus, a burr hole was drilled over the left frontal cortex, and an approximately 5mm craniotomy was performed over the left parietotemporal cortex. A temperature probe was inserted into the left frontal cortex, and body temperature was monitored by a rectal probe (Physitemp Instruments, Clifton, NJ). After brain temperature reached 37.0 ± 0.5°C for 5 minutes, mice were subjected to vertically directed CCI (stereotaxic coordinates of center of impactor tip relative to bregma: anteroposterior = −2.0; mediolateral = +1) using a pneumatic cylinder (Bimba, Monee, IL) with a 3mm flat-tip impounder (velocity, 5.82m/sec; duration, 47 milliseconds; depth, 1.2mm; and driving pressure, 73 psi). Immediately after injury, the bone flap was replaced and sealed with Koldmount cement (Vernon Benshoff, Albany, NY), and the scalp was sutured closed. Isoflurane was then discontinued, and mice were allowed to recover from anesthesia in an oxygen hood for 30 minutes and were then returned to their cages, where they remained under daily observation during the duration of this study. Time-matched shams, in all studies, were subjected to all aspects of the protocol (surgery, anesthesia, craniotomy, recovery) except for trauma; after the craniotomy, the removed bone was reattached using Koldmount cement, and the incision was closed.

Biochemical Analyses

After injury and survival interval, the hippocampus ipsilateral to injury was isolated, frozen on dry ice, and stored at −80°C. Frozen tissue was sonicated in 50mM Tris, pH 8.0, 150mM NaCl, 0.1mM phenylmethyl sulfonyl fluoride, and 0.1mM leupeptin, and centrifuged at 14,000 rpm for 20 minutes. Levels of human Aβ1-40 and Aβ1-42 were assayed in the (Tris-soluble) supernatant, using fluorescent-based ELISA (Biosource, Camrillo, CA), as described previously.[7] Tissue sample protein concentrations were determined using a Pierce BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA). Aβ values are expressed as picogram Aβ per milligram tissue protein.

Immunohistochemistry

Brains were collected from mice perfused transcardially with 30ml of 4% paraformaldehyde made in phosphate buffer (pH 7.4) and postfixed overnight. The brains were cryoprotected in 30% sucrose and sectioned on a freezing microtome at 30μm through the rostrocaudal extent of the injury lesion. Immunohistochemistry was performed as described previously[24] using antiserum generated against synaptophysin (mouse monoclonal, clone SVP-38, lot 045K4828, product S5768, diluted 1:600; Sigma, St. Louis, MO) or F4/80 (rat monoclonal, clone CI:A3-1, product NB600-404, diluted 1:1200; Novus Biologicals, Littleton, CO) and affinity-purified donkey anti–mouse biotinylated secondary (diluted 1:250, synaptophysin; Jackson ImmunoResearch, West Grove, PA), or affinity-purified rabbit anti–rat biotinylated secondary (diluted 1:250, F4/80; Vector Laboratories, Burlingame, CA). Bound antisera were visualized using the Vector ABC Elite kit (Vector Laboratories) and the peroxidase-H_2O_2 reaction with nickel-enhanced 3,3′-diaminobenzidine as the chromogen. There were no immunohistochemistry-labeled structures in sections processed with the omission of the primary antibody except for a diffuse, light signal in the neuropil around the lesion cavity.

Analysis of hippocampal synaptophysin immunoreactivity was performed on three evenly spaced sections through the rostrocaudal extent of hippocampus, approximately 1.5 to 2.5μm caudal to bregma, by calculating the optical density of immunoreactive puncta in the CA1 and CA3 region using the National Institutes of Health (NIH) Image computer program (http://rsb.info.nih.gov/nih-image/). Glial cell activation was assessed by determining the frequency of F4/80-immunoreactive cells that exhibited morphological characteristics of "activated" microglia (stunted enlarged processes and amoeboid shape).

Histopathological Analysis

Tissue sections adjacent to those processed for immunohistochemistry were processed histochemically with cresyl violet acetate (Sigma). Cortical lesion volume was determined by calculating the percentage tissue loss ipsilateral to injury (compared with contralateral) on three Nissl-stained tissue sections through the lesion at the level of the hippocampus, approximately 1.3, 2.3, and 3.3μm caudal to bregma. The cortex was outlined both ipsilaterally and contralaterally using NIH Image; percentage tissue area ipsilateral compared with contralateral was then determined. Similarly, on each tissue section, the hippocampus was outlined, and percentage tissue area was determined on the ipsilateral compared with the contralateral side.

Analysis of Neurological Outcome

ROUND BEAM BALANCE TASK.

Gross vestibulomotor function was assessed using a round beam-balance task in which mice were placed on a quarter-

inch-diameter tube and latency to fall was measured (up to 60 seconds). Baseline assessment (pretesting) was done the day before injury, and the results of five trials were averaged to obtain means and standard errors.

MORRIS WATER MAZE APPARATUS.

The water maze used a 180cm-diameter and 60cm-high metal pool filled with 25°C water to a depth of 28cm. A platform 10cm in diameter and 26cm high (ie, 2cm below the water's surface) was used as the hidden goal platform. The pool was located in a 2.5 × 2.5m room with numerous extramaze cues (eg, shelves, pipes) that remained constant. A video tracking system (Chromotrack; San Diego Instruments, San Diego, CA) was used to record and quantitate the swimming motions of the animals. The tracking system consisted of a charge-coupled device camera, mounted over the pool and attached to a tracking detection unit interfaced to a 386 PC.

HIDDEN PLATFORM TASK.

The hidden platform version of the Morris water maze[27] was used to assess spatial memory acquisition. Five-day acquisition blocks consisted of four daily trials over 5 consecutive days. Mice started a trial from each of the four possible start locations in random order. The location of the platform was held constant for each animal and was varied between animals. The first 2 days consisted of a visible platform task in which the platform was raised 2cm above the surface of the water to control for potential nonspecific deficits in visual and motor function. Mice were given a maximum of 120 seconds to find the hidden platform. All mice were allowed to remain on the platform for 30 seconds before being placed in a heated incubator between trials (4-minute intertrial interval). A single probe trial to determine memory retention was performed on day 5, in which the platform was removed and the percentage time in the platform quadrant was measured by a video tracking system.

Statistical Analyses

Injured or sham-operated mice, treated with simvastatin or vehicle, were compared using one-way analysis of variance with Tukey's post hoc testing for each time point (3 and 7 days) and Aβ species (Aβ40 and Aβ42). Histological and immunohistochemical variables were compared between injured vehicle- and simvastatin-treated mice using two-sample, two-tailed *t* tests. Neurological outcome measures (round beam balance, wire grip, wire score, and Morris water maze) were assessed using two-way repeated-measures analysis of variance and Tukey's post hoc testing. Statistical analyses were performed using the SPSS version 16 statistical package (SPSS, Chicago, IL).

Results

At 3 and 7 days after injury, CCI resulted in a twofold increase in Tris-soluble Aβ40 and Aβ42 peptide levels in the hippocampus of vehicle-treated mice compared with sham-operated control mice (Table). Simvastatin treatment reduced postinjury Aβ concentration to levels that were no longer significantly increased compared with sham-operated control mice. At 7 days after injury, Aβ levels in CCI/simvastatin-treated mice were also not significantly different from sham control mice and were significantly lower than those in CCI/vehicle-treated mice.

We next examined histological outcome after CCI by evaluating cortical and hippocampal tissue loss, density of presynaptic terminals, and microglial activation in simvastatin-treated versus vehicle-treated mice. Although sham-operated mice exhibited a small amount of cortical compression without tissue necrosis and loss, mice subject to CCI developed a large cortical necrotic hole and compression to the underlying hippocampus (Fig 1). A significant reduction in hippocampal tissue loss after injury was observed in the simvastatin-treated group compared with vehicle-treated mice, whereas cortical tissue loss was not significantly different between the two groups (see Fig 1).

To assess the degree of hippocampal synaptic integrity in this injury model, we measured intensity of synaptophysin immunoreactivity by optical density in the hippocampal CA3 (stratum lucidums [sl]) and CA1 (stratum radiatum [sr]) regions. These regions were chosen as areas of secondary injury (not subject to direct mechanical injury). They are amenable to analysis as relatively discrete anatomic structures and are important components of the hippocampal trisynaptic pathway. Compared with sham control mice, we observed that synaptophysin immunoreactivity in CA3sl was reduced in CCI/vehicle-treated mice, but not in CCI/simvastatin-treated mice ($p < 0.05$; Fig 1), suggesting that synaptic integrity was preserved as a result of simvastatin intervention. In CA1sr, sham-operated animals exhibited robust synaptophysin immunoreactivity surrounding and outlining dendritic processes (see Fig 1A). Synaptophysin immunoreactivity was greatly reduced in CA1sr of CCI/vehicle- and CCI/simvastatin-treated mice, and there were no apparent differences in the two injured groups (see Figs 1B, C). Thus, unlike in CA3sl, in CA1sr simvastatin was not protective against synaptic loss after CCI.

The extent of microglial activation was examined in the CA3 and CA1 hippocampal fields (Supporting Fig 3A). Compared with lightly F4/80-immunoreactive cells on the contralateral (uninjured) side and in sham-operated animals (see Supporting Figs 3C, D), the F4/80-immunoreactive cells on the side ipsilateral to injury in CCI/vehicle-treated animals were more robustly labeled and exhibited morphological characteristics of reactive microglia (see Supporting Figs 3E, F). In particular, these cells had enlarged somas and short, thickened, darkly stained processes. In contrast, CCI/simvastatin-treated mice had lightly F4/80-immunoreactive microglia on the side ipsilateral to injury (see Supporting Figs 3G, H); these cells were sim-

Table. **Hippocampal Aβ Peptide Levels 3 Days and 7 Days after Sham Surgery or Controlled Cortical Impact (CCI) in $APP^{NLh/NLh}$ Mice Administered Either Vehicle (Veh) or 3mg/kg Simvastatin (Sim).**

Survival interval	ANOVA	Aβ Peptide level	ANOVA *p*-value	Post Hoc	Post-Hoc *p*-value
3 days Aβ40					
	Sham	6.02 ± 1.39	0.011	Sham < CCI/Veh	$p < 0.01$
	CCI/Veh	10.33 ± 2.17			
	CCI/Sim/	7.85 ± 2.32			
3 days Aβ42					
	Sham	8.18 ± 4.74	0.003	Sham < CCI/Veh	$p < 0.01$
	CCI/Veh	16.17 ± 2.52			
	CCI/Sim/	12.28 ± 1.54			
7 days Aβ40					
	Sham	5.32 ± 1.62	0.002	Sham < CCI/Veh	$p < 0.01$
	CCI/Veh	12 ± 3.1		CCI/Sim < CCI/Veh	$p < 0.05$
	CCI/Sim/	8 ± 1.68			
7 days Aβ42					
	Sham	9.03 ± 3.63	0.002	Sham < CCI/Veh	$p < 0.01$
	CCI/Veh	17.23 ± 3.42		CCI/Sim < CCI/Veh	$p < 0.05$
	CCI/Sim/	11.3 ± 2.1			

Values are expressed as pg/mg protein. Groups (Sham, CCI/Veh and CCI/Sim) were compared using one-way ANOVA and individual comparisons were performed using Tukey honestly significant difference (HSD) post-hoc testing.
Aβ = β-amyloid.

ilar in appearance and immunostaining intensity to those on the contralateral (uninjured) hemisphere.

We next examined whether the protective effects of simvastatin treatment on histological outcome after injury were reflected in improved functional outcome as measured by the Morris water maze (MWM) spatial learning task (days 10–14) and memory retention task (probe trial) at 14 days after injury. The spatial learning data indicated that the injury groups had significantly longer latencies to find the hidden platform than the sham groups (Fig 2A). Simvastatin treatment did not alter spatial learning after TBI or sham surgery. The probe trial data indicate that the CCI/vehicle-treated mice performed significantly worse compared with sham operates in relative place memory retention in MWM testing (see Fig 2B). In contrast with spatial learning performance, injured mice treated with simvastatin, however, performed as well as sham operates, and significantly better than their CCI/vehicle-treated counterparts (see Fig 2B). There were no statistically significant differences among groups in swim speed, turning ratio and path length, (Supporting Table 2) during the probe trial or in motor performance evaluated by round beam balance (see Fig 2C) or wire grip test (data not shown) before MWM testing. This indicates that greater motor impairment in the CCI/vehicle-treated group did not account for the observed differences in the MWM trial.

Discussion

This study demonstrates that simvastatin, administered at a clinically relevant time interval (3 hours) after CCI injury, blunts trauma–induced increases in Aβ peptide levels in the hippocampus. This effect was a part of overall histological and neurological improvement after injury, including preserved hippocampal tissue integrity and synaptic connections, reduced microglial activation, and improved performance in the MWM; these latter observations add further translational applicability of this model to a clinical setting. Our findings are important in light of rapidly accumulating evidence that soluble Aβ species can impair hippocampal synaptic function.[28] However, they also raise the compelling question whether, in human brain injury, modulation of Aβ levels during the acute injury period would prevent a potential cascade of Aβ-related neuronal dysfunction. In addition to improving acute neurological recovery after TBI, the reduction in Aβ load by simvastatin intervention may also reduce the risk for development of AD later in life. The observed Aβ-reducing effect of

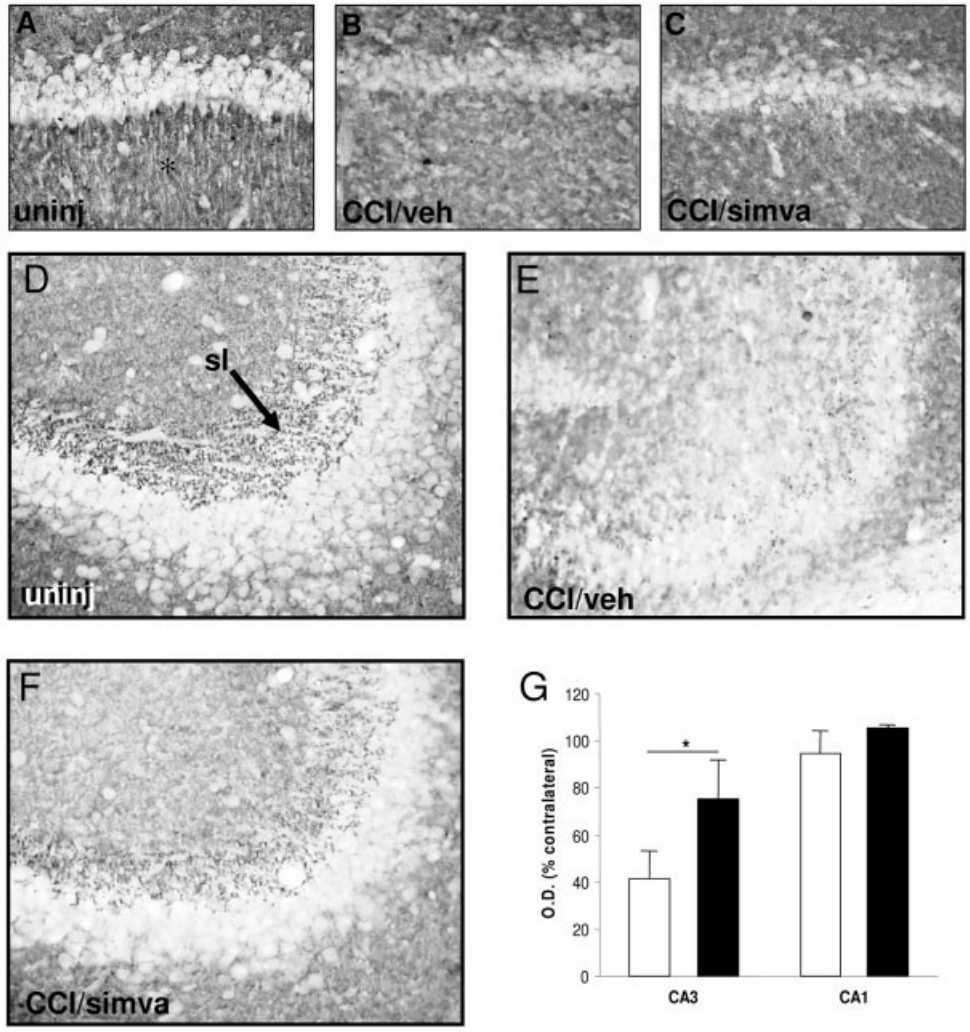

Fig 1. Synaptophysin-immunoreactive profiles in hippocampal CA1 stratum radiatum (A–C) and CA3 stratum lucidum (D–F, arrow) in mice 14 days after controlled cortical impact (CCI) and either vehicle (B, E; CCI/veh) or simvastatin (C, F; CCI/simva) treatment, and in naïve mice (A, D). Punctate dendritic labeling observed in the CA1 pyramidal cell region in uninjured (uninj) animals (asterisk) *is reduced equally in injured/vehicle-treated and injured/simvastatin-treated groups. In contrast, injury resulted in considerable loss of punctate immunolabeling in the stratum lucidum and pyramidal cell layer of CA3, which was attenuated in animals treated with simvastatin. The results of quantitative assessment of optical density (OD) of synaptophysin immunohistochemistry are shown in (G) (*$p < 0.05$*, two-tailed Student's* t *test).* White bars *represent vehicle treatment;* black bars *represent simvastatin treatment. Scale bar =* $25\mu m$*.* * = $p < 0.05$.

simvastatin treatment in injured $APP^{NLh/NLh}$ mice is in agreement with studies that reported Aβ-lowering effects using higher doses of simvastatin in intact (uninjured) animals carrying the human Aβ sequence (ie, guinea pigs[29]) and young human APP overexpressing (APP2576) transgenic mice.[30]

Statins are cholesterol-lowering drugs; thus, it is reasonable to consider that the effects observed in our study are due to changes in total brain cholesterol levels or cell membrane cholesterol distribution. However, this is not likely because only chronic administration of statins (eg, longer than weeks) results in redistribution of intracellular and membrane cholesterol, and changes in secretase activity.[31–33] These observations are clinically relevant, because inhibition of cholesterol synthesis in the central nervous system after injury could affect long-term recovery, membrane reparation, and synaptogenesis. Statin treatment may also alter levels of apolipoprotein E,[34] which, in turn, may modulate Aβ[35] or cellular cholesterol levels, or both. Future studies using more chronic time points and prolonged treatment after injury will determine statin effects on brain cholesterol and Aβ levels during long-term recovery from TBI.

The effects of acute simvastatin treatment on Aβ levels may be the result of an attenuation of the

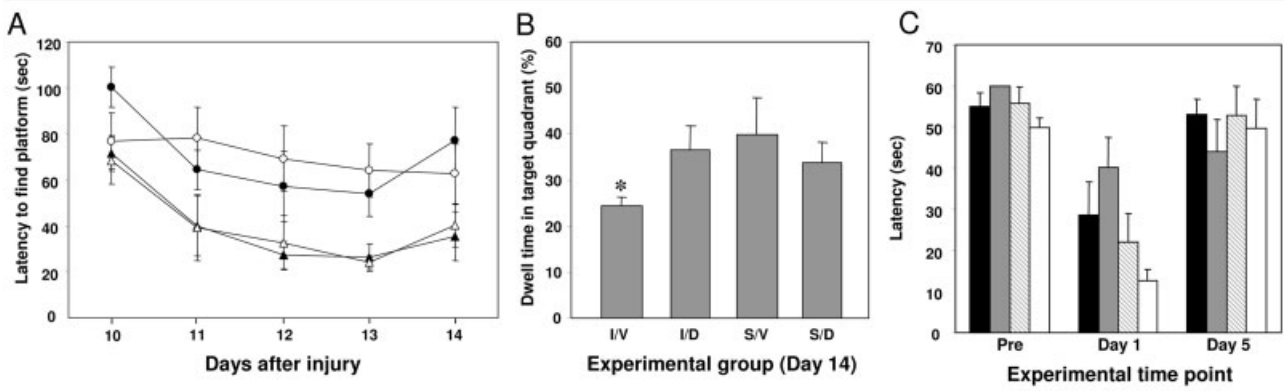

Fig 2. Results of Morris Water Maze (MWM) memory acquisition (A) and retention (B) MWM memory retention on days 10 to 14 after controlled cortical impact (CCI) or sham surgery with or without statin intervention. Although the two injured groups performed significantly worse than their sham counterparts in the memory acquisition phase of the trial, they did not differ between themselves. However, in the probe trial of memory retention, the injured simvastatin-treated group performed better than the injured vehicle-treated group, and equally well as both sham groups. There were no differences between the four groups in the round beam balance test of vestibular function (C) Round beam balance assessed on days 1 and 5 after injury (days 2–4, data not shown). (A) Black circles *represent I/V;* white circles *represent I/D;* black triangles *represent S/V;* open circles *represent S/D. (C)* Black bars *represent I/V;* gray bars *represent I/D;* hatched bars *represent S/V;* white bars *represent S/D.*

postinjury inflammatory response. This could involve reductions in the levels of the inflammatory cytokine interleukin-1β, which, in turn, may alter APP metabolism.[36–38] The reduction in microglia activation may also contribute to improved outcome after injury. Although no immunohistochemical evidence of Aβ accumulation in microglia was found in a previous study using the same mouse model,[24] microglial uptake of soluble (oligomeric) Aβ may still occur, inducing cytokine release.[39] Microglial activation induced by intracerebroventricular injections of Aβ is attenuated by atorvastatin treatment[40]; this is particularly relevant to this study, because inhibition of hippocampal long-term potentiation by Aβ is, in part, due to microglial activation.[41] In addition, glial cell activation is associated with increased expression of enzymes within the γ-secretase complex,[42] and modulation of isoprenoid synthesis can influence APP metabolism.[43,44] Whether simvastatin-induced reduction in Aβ production after TBI occurs in parallel with attenuated production of the Kunitz protease inhibitor–containing form of APP, which is increased after brain injury,[45] and increased production of secreted nonamyloidogenic soluble APP alpha (sAPPα) fragments, which may be important for neuronal repair and synaptogenesis,[46] is currently under investigation. These issues will be particularly relevant in the assessment of chronic survival periods where APP production may remain increased as the brain undergoes repair and rewiring.

The positive histological and behavioral outcomes of simvastatin intervention in our TBI investigation are in accord with Chopp and colleagues' brain injury studies using rats.[20,47,48] Statins have also proved protective in several other acute brain injury paradigms, including ischemia,[17] excitotoxicity,[49–51] intracerebral hemorrhage,[52] and thrombosis,[53] all of which can contribute to secondary neuronal injury and cell death after TBI.[54] The protective effect of statins in multiple neuronal injury models likely reflects their pleiotropic actions in the central nervous system.[55] In the case of simvastatin, these include decreased production of isoprenoids,[56] increased Akt phosphorylation,[57] reduced integrin-dependent leukocyte adhesion,[55] induction of endothelial nitric oxide expression,[18] and improved cerebral perfusion after brain injury.[58,59] Accordingly, the results of our Aβ analysis may be interpreted in the context of the overall improved hippocampal neuron viability, which could result in fewer stressed or injured neurons that would likely release Aβ. This could alter the total protein-to-Aβ ratio, resulting in less pronounced protein loss and relatively lower values of Aβ by ELISA. However, our previous immunohistochemical investigation using the same mouse injury model demonstrated prominent intraneuronal Aβ42 immunoreactivity after injury.[24] This indicates that, in biochemical assays of tissue homogenates, the observed CCI-induced Aβ increases are not artifactual (eg, because of loss of other proteins). Furthermore, biochemically detected modulations in Aβ levels by a postinjury caspase inhibition were corroborated by Aβ immunohistochemistry.[24] Alternatively, reducing Aβ may prevent neuronal damage and behavioral deficits after injury, as this molecule has toxic effects on neuronal viability[60] and synaptic plasticity[28] and increased soluble Aβ concentration impairs long-term

potentiation at the level of the synapse in the hippocampus.[61–63]

In conclusion, our results demonstrate that simvastatin therapy prevents postinjury increases in hippocampal Aβ levels, ameliorates structural and behavioral deficits, and could be effective as a therapeutic agent acutely after human brain injury. The effects of acute and chronic statin treatment on outcome in the months and years after injury, and their potential for decreasing the risk for development of AD later in life, will be an important avenue of future research.

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